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Assessment of some Morphometric Parameters in Ram Sperm Correlated with the Collection Method

Dana TAPALOAGA[a]*, Paul-Rodian TAPALOAGA[a]

[a]*University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, Bucarest-1, Romania*

Abstract

It is well known that assisted reproductive techniques are implemented and used worldwide in livestock production, having as aim to allow high genetic animals to produce more descendants in a shorter period of time than possible naturally. They have enabled animal breeders to optimize production and reproduction efficiency of their animals by improving selective traits and accelerating the genetic progress. Artificial insemination techniques in sheep increase the selection differential, being one of the main biotechnologies that are used extensively in the animal livestock.

The present research has in view to assess the influence of collection techniques upon some morph metric parameters of the ram sperm. There were analyzed 10 native purebred and crossbred rams from different age category. The semen collection was made by artificial vagina and with the aid of electro ejaculator. At every semen collection, there were recorded the main morphologic features of sperm and also the primary morph metric head sperm indices: head length, head width, head area and head perimeter. The influence of the collection technique was in view along two years, studying the dynamics of the analyzed sperm indices through the computer image analysis.

Descriptive statistics were performed on the recorded data to determine normality. Statistical analysis was performed as per standard statistical methods. The results reveal the fact that there were no morph metric differences between the two collection methods. The lack of significant differences indicated that other factors may affect the reproduction efficiency between the two methods. The study clearly demonstrates that there is a variation in reproductive parameters in the rams which could be studied at the molecular level to unveil any genomic markers associated with low fertility and/or infertility. The results obtained for this part of the overall study emphasizes further research to correlate morph metric traits of ram sperm with conception rate of ewes to determine whether morph metric traits need to be included in sperm quality tests for artificial inseminations.

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Keywords: sperm collection, artificial vagina, electro ejaculator.

* Corresponding author. Tel.: +4021.318.25.64; Fax: +4021-318-0466.
E-mail address: drtapaloaga@yahoo.com

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1. Introduction

The more and more increased potential of assisted reproduction techniques helps sheep producers to optimize the production and reproductive efficiency of their flocks (Raducuta et al., 2015). The application of these techniques, such as artificial insemination (AI) and multiple ovulation and embryo-transfer (MOET) gives arguments which allow them to produce lamb and muttons more cost-efficiently. That is why they contribute significantly, if applied properly, to optimize and ensure cost-efficient livestock production (Al. Dulaimi, 2016).

2. Materials and Methods

The study was conducted on the Laboratory of Animal Reproduction, Animal Sciences Faculty, University of Agronomical Sciences and Veterinary Medicine, Bucharest, Romania.

All animal care and procedures used in the study were performed without affecting environment and animal's welfare.

A total of 10 rams (*Ovis aries*) aged 2 to 6 years were used in the study. The rams are part of a research flock established and maintained at a private farm; they were maintained according to ethically approved husbandry practices, under uniform nutritional conditions.

The studied rams were one of the native Romanian breed and also crossbred. The studied rams were firstly exposed to the breeding soundness evaluation. The general health of the ram was evaluated and any abnormalities recorded. Especially the eyes, feet, legs and penis were observed for any defects that could interfere with the breeding process. The body condition was observed and a score noted. The testes and epididyms were palpated being known that the impairment of one testicle with scar tissue, or abnormally small size will almost certainly reduce the breeding capacity and endurance of that ram, even if the semen parameters are normal (Tapaloagă, P. 2003, Tapaloaga Dana 2004, Al. Dulaimi, 2015). The measurement of the scrotal circumference gave a good indication of breeding endurance and has also been correlated in sheep with age at development of puberty (Tapaloagă, P. 2003, Tapaloaga Dana 2004, Al. Dulaimi, 2015, Al. Dulaimi, 2016, Boshoff Ninja Hettie, 2014). Ram lambs of less than 30 cm and adult rams of less than 31 cm were not approved as acceptable breeders, so the same in the present research. However, recognize that scrotal circumference may be greatly decreased by recent weight loss as well as by season of the year (smallest in the spring and early summer). For measuring, we pulled the tape tight on the scrotum at its area of greatest circumference, and then let out some slack in the tape until it stops at a consistent tension.

All the data collected during breeding soundness examination were recorded into a data collection file. The chosen rams were randomly allocated to the two groups, and semen was collected daily over a period of four weeks from each of these two groups. Semen was thus collected from each ram, once a week, with a total of twenty analyzed collections per ram for the period of the study. On collection days, the rams were brought in from the pasture, and placed in individual indoor pens, until used for semen collection. Semen collection was performed in a separate pen, where a heat ewe was restrained as the dummy/teaser for collection. There were made semen collections with the aid of the artificial vagina and the electro-ejaculator.

The AV was prepared beforehand to ensure that the collection conditions optimized the viability of the semen samples. The temperature in the AV ranged between 42-45^{0}C, and was monitored between each sample collection procedure. Prior to collection, care was taken to pre-warm all equipment (collection tube, AV inner, microscope slides and pipette tips) to 37ºC to avoid cold-shock damage to the sperm during collection and processing. During collection, care was taken to prevent contamination of the samples (Tapaloagă, P. 2003, Tapaloaga Dana 2004).

There were carried out morph metric determinations with the aid of Full ISAS CASA System, composed by ISAS software, UB203i phase contrast microscope with epi-fluorescence and heated stage, heated stage controller, camera and computer was used for sperm morphometry. With the aid of ISAS v1 in our study, the morph metric features of sperm cells were performed from stained samples. The data were recorded at the Faculty of Animal Science, U.A.S.V.M. Bucharest. All tables and graphs were prepared in Microsoft Excel (2010). All data collected with the ISASv1 were converted from *.mot file format to the Excel file format prior to analysis. All results were expressed as the mean ± standard error of the mean. Findings were considered statistically significant when $p<0.05$. There were analyzed especially the head size or primary parameters (area, length, width and perimeter) and the results were compared with the other in the literature in the field.

3. Results and Discussions

There were carried out morph metric determinations with the aid of Full ISAS CASA System. The main primary morph metric parameters measured by ISASv1 in the present study were the ones related to the size of the sperm head: the length of the head, the width of the head, the area and the perimeter of the head. The following tables and charts show the main values recorded and the data were grouped depending on the semen collecting method.

The mean values of the length of the sperm head in the studied livestock recorded values ranged between 7.35±0.03 µm in ram no.2 and 7.84±0.02 µm in ram no.5. Comparatively, in 2010, Gravance CG, Champion ZJ and Casey PJ, in the study *Computer-assisted sperm head morphometry analysis (ASMA) of cryopreserved ram spermatozoa*, quoted by Hussein Al Dulaimi in his doctoral thesis (Al. Dulaimi, 2016) highlighted the accuracy of ASMA procedures in sperm assessment and found that sperm head length was 8.08 µm. Also, Ninja Hettie Boshoff, in her MSc. Thesis at University of Stellenbosch, South Africa, *The influence of genotype on sperm motility and sperm head morphometry of Merino (Ovis aries) sheep*, quoted by Hussein Al Dulaimi (Al. Dulaimi, 2016, Boshoff Ninja Hettie, 2014**)** found that the length of sperm head in a High Line of Merino rams ranged between 7.92 – 8.70 (2.89) with a mean of 8.36 ± 0.07 microns and in a Low line of Merino rams ranged between 7.73 – 8.70 (3.03) with a mean of 8.40 ± 0.07 microns. Meanwhile, Hussein Al Dulaimi and col. (2015) found that the mean values recorded in their study varied between 7.4276±0.029537 µm and 7.9064±0.016237 µm. The mean values of the length of the sperm head in the studied livestock depending on the collecting method of semen expressed in µm are shown in table 1.

The recorded data reveals the superiority of the length of the sperm head in favour of the electro ejaculation collection method, beside the artificial vagina collection method. The recorded mean values are 7.68±0.03 µm, respectively, 7.71±0.001 µm, in both cases with a low variability.

Table 1. Mean values of the length of the sperm head (µm)

Semen collection method	X ± s_x(µm²)	± s	V %
Artificial vagina (n = 150)	7.68±0.03	0.18	2.05
Electro-ejaculation (n = 50)	7.71±0.001	0.14	2.72

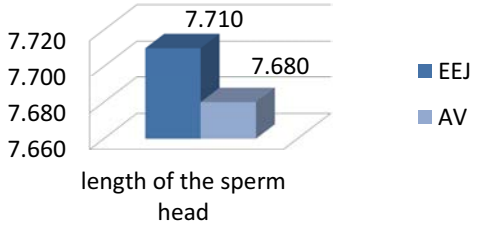

Fig. 1. Dynamics of mean values of sperm head length

Figure 1 presents the little difference mentioned above. Little superiority of the length of the sperm head in favour of the electro ejaculation collection method, beside the artificial vagina collection method was noticed. Both cases recorded low variability. There were not recorded differences between the data obtained by grouping the samples upon the collection method, the AV method and the EEJ method, $p < 0.8180$ (non significant differences).

The mean values of the width of the sperm head in the studied livestock depending on the collecting method of semen are presented in table 2. It could be seen that in the samples come from electro ejaculation method of collection, the width of sperm head was higher, 4.68±0.05µm, than the width of sperm head come from artificial vagina method of collection, 4.42±0.03 µm.

Hussein Al Dulaimi and col. (2015, 2016) found that the mean values recorded in their study varied between 4.512121 ± 0.012822 µm, than the width of sperm head come from artificial vagina method of collection, 4.490575±0.008384 µm. In 2010, Gravance CG, Champion ZJ and Casey PJ, in the study *Computer-assisted sperm head morphometry analysis (ASMA) of cryopreserved ram spermatozoa*, quoted by Al Dulaimi (2016, 2015) highlighted the accuracy of ASMA procedures in sperm assessment and found that sperm head width was 4.80 microns.

Table 2. Mean values of the width of the sperm head (μm²)

Semen collection method	X ± s_x(μm²)	± s	V %
Artificial vagina (n = 150)	4.42±0.03	0.12	4.31
Electro-ejaculation (n = 50)	4.68±0.05	0.21	2.81

Figure 2 presents the dynamics of the mean values of sperm head width depending on collection method, showing the superiority of the electro ejaculation method beside the artificial vagina method, with 5.55%.

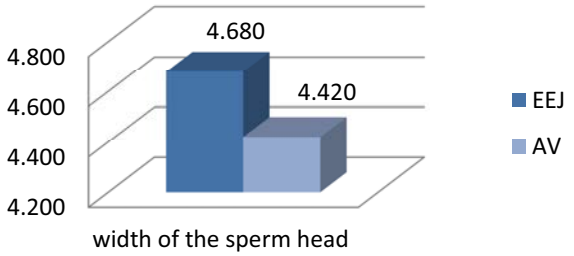

Fig. 2. Dynamics of mean values of sperm head width

The area of the sperm head was assessed with the aid of ISAS v1 soft upon the base of the captured images of the sperm cells in the studied livestock. The mean values of the area of the sperm head in the studied livestock ejaculates expressed in μm² are presented in the following table and chart. It could be notice in the table 3 that the lowest value of the area of the sperm head was recorded in ram number 3, 31.42±0.22 μm² and the highest recorded value in ram number 9, 38.19±0.15 μm². Comparatively with our values, the literature in the field published the paper of Gravance CG, Champion ZJ and Casey PJ, *Computer-assisted sperm head morphometry analysis (ASMA) of cryopreserved ram spermatozoa,* quoted by Al Dulaimi (2015), where the authors recorded a mean value of sperm head area of 29.13 μm², this value being lower than our mean and also our minimum range.

Also, in 2014, Ninja Hettie Boshoff found that the area of the sperm head in Merino rams ranged between 36.48 – 43.35 (4.90) μm² with a mean of 40.56 ± 0.51 μm² (Al. Dulaimi, 2016, Boshoff Ninja Hettie, 2014). Meanwhile, Hussein Al Dulaimi and col. (2014, 2015) found that the mean values recorded in their study varied between 32.69635±0.265664 μm² and 37.99325±0.130899 μm².

The mean values of the area of the sperm head in the studied livestock depending on the collecting method of semen are presented in table 3. It may notice almost the same values of the sperm head area depending on the collection method.

The mean value of the area of the sperm head was higher in case of electro ejaculation method, with a value of 36.38±0.17 μm² than the one reported in case of artificial vagina collection method, the value being 34.46±0.053 μm², the difference between the two methods being 5.27%, p< - 1.135 (non significant differences).We could compare our recorded data with the ones published by Hussein Al Dulaimi and col. (3,4), who found that the mean values recorded in their study was 35.2575±0.128163 μm² in case of electro ejaculation and 35.0604±0.081782 μm², in case of artificial vagina collection method, the difference between the two methods being very low, only of less than 0.19 μm².

Table 3. Mean values of the area of the sperm head (μm²)

Semen collection method	X ± s_x(μm²)	± s	V %
Artificial vagina (n = 150)	34.46±0.053	1.42	2.54
Electro-ejaculation (n = 50)	36.38±0.17	1.31	3.31

The Figure 3 below shows the discussed difference in the recorded size of sperm head.

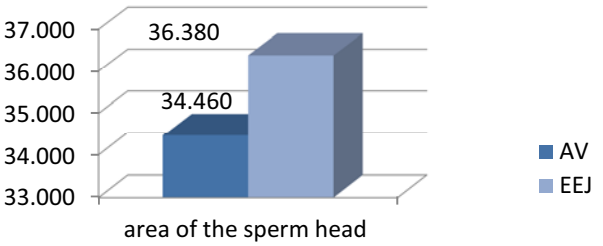

Fig. 3. Dynamics of mean values of sperm head area depending on collection method (μm²)

The sperm head perimeter was the last primary index of sperm size assessed in the present study.

It may notice that the values of the sperm head perimeter ranged between 17.25±0.03 μm in ram 1 and 19.14±0.031957 μm in ram 8.

Analyzing the values of the sperm head perimeter in relation to the collection method, it may notice from table 4 and chart 4 that the values reported from the measurement of the sperm head obtained by electro ejaculation method were higher than the ones reported from the measurement of the sperm head obtained by artificial vagina method, even the differences were not so large.

The recorded mean value was 17.54 μm in case of AV and 18.01 in case of EEJ. The special literature published the values reported by Gravance CG, Champion ZJ and Casey PJ, in 2010, where the authors recorded a mean value of sperm head perimeter of 23.93 microns, this value being higher than our mean and also our maximum range. Also,comparatively to our values, in 2014, Ninja Hettie Boshoff, found that the perimeter of the sperm head in Merino rams ranged between 17.93 – 19.79 (2.87) μm with a mean of 19.12 ± 0.14 μm. Hussein Al Dulaimi and col. (2015), found that the mean values recorded in their study were 17.98292±0.063209 μm in case of EEJ and 17.93272±0.034605 μm in case of AV.

Table 4. Mean values of the perimeter of the sperm head (μm)

Semen collection method	X ± s_x(μm²)	± s	V %
Artificial vagina (n = 150)	17.54±0.04	0.55	3.22
Electro-ejaculation (n = 50)	18.01±0.05	0.61	3.52

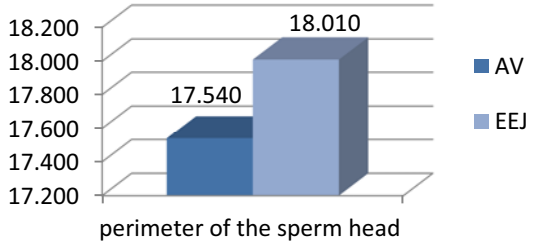

Fig. 4. Dynamics of mean values of sperm head perimeter

4. Conclusions

The mean values of the length of the sperm head ranged between 7.35±0.03 μm and 7.84±0.02 μm. Little superiority of the length of the sperm head in favour of the electro ejaculation collection method, beside the artificial vagina collection method was noticed. The recorded mean values are 7.68303±0.03 μm, respectively,

7.71±0.001 μm, in both cases with low variability. There were not recorded differences between the data obtained by grouping the samples upon the collection method, the AV method and the EEJ method.

The width of sperm head was higher n case of EEJ, (4.68±0.05 μm), than the width of sperm head come from artificial vagina method of collection (4.42±0.03 μm), but there were not recorded differences between the methods.

The mean value of the area of the sperm head was higher in case of electro ejaculation method, with a value of 36.38±0.17 μm² than the one reported in case of artificial vagina collection method, the value being 34.46±0.053 μm², the difference between the two methods being very low, so they did not differ from the statistically point of view.

The values of the sperm head perimeter ranged between 17.25±0.03 μm and 19.47±0.03 μm. Analyzing the values of the sperm head perimeter in relation to the collection method, it may notice that the values reported from the measurement of the sperm head obtained by electro ejaculation method (18.01μm) were higher than the ones reported from the measurement of the sperm head obtained by artificial vagina method (17.54 μm), but without any statistic differences.

The results obtained for this part of the overall study emphasizes further research to correlate morph metric traits of ram sperm with conception rate of ewes to determine whether morph metric traits need to be included in sperm quality tests for artificial inseminations.

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